

Sexual selection and sex ratio in

flies and moths

Submitted by *William Larner*, to the University of Exeter as a thesis for the
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(Signature)WILLIAM LARNER.....

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Chapter 1: General introduction

Sexual selection

Darwin's theory of sexual selection (1871) is based on differences in reproductive success both within and between the sexes. These differences are caused by competition over access to mates or choice of particular mating partners. Sexual reproduction involves the combination of genetic material from males and females. The two sexes have defining gamete properties; males tend to invest in numerous small, mobile sperm, whereas females tend to produce fewer large, nutritious eggs (Anderson 1994). As females generally invest more resources in gamete and offspring production than males, their reproductive rate is considerably lower than that of males (Trivers 1972). This means that receptive females are usually in short supply, leading to fierce competition between males over access to females (Parker et al. 1972). Consequently, males often achieve greater reproductive success by competing with other males to access as many females as possible. Variance in male reproductive success is, therefore, largely determined by their mating success (Wade 1979). Female reproductive success on the other hand is largely determined by the amount of nutrients available for egg and offspring production (Trivers 1972). As females generally invest more in each individual offspring (Robert 1972) they benefit from being the choosier sex and exercise this choice over their mating partner to preferentially mate with the 'best' male. Therefore, because male and female routes to reproductive fitness are very different, sexual selection is generated both within and between the sexes.

In developing the theory of sexual selection, Darwin (1871) accounted for the

evolution of secondary sexual characteristics that were unlikely to aid survival and which, at times, may be directly detrimental to the bearer. Secondary sexual characteristics are not directly part of the reproductive system, but are traits used in the competition over mates or to appear more attractive to a mating partner (Anderson 1994). Such traits vary between individuals and can include differences in size, colour, ornamentation and behaviour. Examples of secondary sexual traits include large antlers in male deer, which result from male-male competition (Clutton-Brock 1988), or elaborate nest building in bower birds (Diamond 1987; 1988; Collias and Collias 2014) and enhanced colouration in male guppies (Kodrick-Brown 1985; Auld et al. 2016), which result from female preference for good quality nest and/or for mating with the best quality partners.

Mate choice

Mate choice also greatly interested Darwin as it can result in extreme sexual dimorphism, such as species with dull coloured females and brightly coloured males. Bright colouration can be costly to males as it may significantly increase their chance of predation. However, female preference for more colourful males increases overall male mating success (Auld et al. 2016) and, therefore, the reproductive benefits outweigh the risks from predation. Fisher (1930) outlined how female preference for male characteristics could evolve in terms of genetic benefits. He proposed that as females choose males with more elaborate characteristics, the offspring of these matings result in individuals with similar characters (sons) and similar preferences (daughters). The 'good genes' hypothesis suggests that females choose traits that are

honest indicators of the male's ability to pass on genes that are likely to increase the survival or reproductive success of her offspring. This process creates a positive feedback between genes for preference and traits, fuelling additional increases in the mean phenotypic value for that sexual character and creating what Fisher called "runaway sexual selection" and 'sexy sons'. This process is suggested to be responsible for the evolution of extreme tail length in widowbirds. Males with extremely long tails have higher reproductive success than males with normal or reduced tails, as females prefer them. The hypothesis that intra-sexual competition among males maintained the long tail phenotype is not supported, as males with shorter tails hold their territories just as successfully as long-tailed birds (Anderson 1992; Lovette 2016). Instead, this finding suggests that female mating preference for long-tailed males maintains extreme tail length in widowbirds, supporting the Fisher process of sexual selection.

Males can also exhibit choice of females. Male mate choice has been reported in a variety of taxa, mostly in insects (Gage & Barnard, 1996; Wearing-Wilde 1996; Bonduriansky 2001), but also in birds (Hill 1993; Heinig et al. 2014), fish (Amundsen and Forsgren 2001) and amphibians (Arak 1983; Eddy et al. 2016). Theory predicts that male mate choice should most commonly be observed in traits that maximise a male's expected reproductive success from each mating. For example, male water striders have been shown to copulate for longer with large females (Rowe and Arnqvist 1996). These longer copulations are most likely a result of a preference for larger females that are more fecund (Honěk 1993). Selection for male choosiness is expected to be strongest when there is greater variance in female body size

and, therefore, female fecundity. Equally, male mate choice is also expected when mating is costly to males, for instance males may provision resources to females, which infers direct benefits for each female mating. In such mating systems the benefits to females, such as nuptial gifts, can be a costly mating investment for males. In such systems it would be predicted that males who invest highly in nuptial gifts will mate with larger females, which will yield higher fertilisation returns. In contrast, selection for male choosiness is predicted to be weaker in systems where there is little variance in female fecundity and when mating is less costly for males.

Female multiple mating (polyandry) is widespread in nature and can be associated with direct and/or indirect benefits to females. Direct benefits of polyandry include fertility assurance and nutrient donations. For example, the duration of copulation in female scorpionflies is correlated with the size of nuptial gifts (prey) presented by the male at mating (Gwynne 2008). Nuptial gifts provide nutritional benefits to females mating with multiple males, as the more males a female mate with, the more nuptial gifts she receives. Equally it has been shown that mating with multiple males increases the egg-hatching success of female scorpionflies when compared to monandrous females that mated twice with the same male (Engqvist 2006). This may simply be due to sperm limitations, as males can suffer from sperm depletion, whereas a male who is mating for the first time can maintain high fertility. In these situations female multiple mating will increase a females' overall reproductive success. Even in socially monogamous birds, females often engage in extra-pair copulations. It has been shown in blue tits (*Parus caeruleus*), that extra-pair copulations occur more frequently when females are paired with low quality

males (Kempenaers et al. 1997; Schlicht and Kempenaers 2013). Low quality, cuckolded males were also shown to have lower over-wintering survival than high quality males. Female multiple mating meant that extra-pair young were more likely to survive in this situation than within-pair young. These results support the idea that female blue tits engage in extra-pair copulations to obtain good genes for their offspring.

By mating with multiple males, it is suggested that females can increase their reproductive success through selection of sperm; preferring sperm of males with high genetic quality (Birkhead et al. 1993). Consistent with this hypothesis, female chickens can eject sperm from subordinate males whilst maintaining sperm from dominant individuals (Pizzari and Birkhead 2000). There is also evidence in weevils, which indicates that females can control sperm movements from multiple males within their reproductive tract (Villavaso 1975; Orr and Brenna 2015). Therefore, a number of different mechanisms that allow females to influence paternity exist, both pre- and post-copulation, and it is increasingly clear that female choice plays an important role in sexual selection.

Mate competition

Variation in mate number among males reflects variation in male-male competitive ability, as well as variation in male attractiveness to females. Female choosiness can lead to elaborate male ornaments (used to attract females), as discussed above. Whereas male-male competition can favour

the development of weapons. For example, in the Hercules beetle (*Dynastes Hercules*) larger males normally invest in the largest or most complex weapons, whereas smaller males will have excessively reduced weapon size. This size difference is due to the higher cost to a small male of developing a large weapon, both in terms of energetic investment and in terms of severe injury during competition with other (larger) males. Therefore, such traits act as honest indicators of male quality, both prior to and during physical combats (Miller 2013).

Male-male competition can also occur post-mating, through sperm competition. Polyandry is widespread in nature and results in competition between males' ejaculates for fertilisation of the female's ova (Parker 1970). The prevalence of sperm competition across taxa can be demonstrated by a positive association between increased testis size and sperm competition risk in many animals, including primates (Harcourt et al. 1981), butterflies and moths (Gage 1994; Morrow & Gage 2000), birds (Møller 1988), and bats (Hosken 1998). Increased testis size leads to a greater ability to produce large ejaculates, which can determine paternity success in sperm competition, as producing relatively more sperm is advantageous (Birkhead et al. 2008). For example, Gage and Morrow (2003) showed that across species of butterflies, males produce relatively more sperm as the risk of sperm competition increases, and tend to achieve higher fertilisation success under sperm competition.

Sperm competition promotes traits that increase male fertilisation success (Birkhead et al. 2008). Even though males produce many sperm, their sperm

production is not limitless; there are energetic costs to producing sperm. For example in *Drosophila*, longer sperm are superior at displacing, and withstanding displacement by, shorter competitor sperm (Miller and Pitnick 2003). Therefore, males would be expected to strategically ejaculate their sperm in order to maximise fertilisations. However, there are substantive developmental and longevity costs associated with longer sperm, (Lüpold et al. 2016). In other words, males should only produce more sperm when it increases their chances of fertilisations. For instance, male rats (Bellis et al. 1990) and beetles (Gage and Baker 1991) ejaculate more sperm when there are rivals present, and this may translate into higher paternity success for males that produced a higher number of sperm. This finding was also reported in the fruit fly (*Drosophila melanogaster*), where flies exposed to rival males prior to mating increased their ejaculate investment (measured as mating duration). These males achieved significantly higher paternity share, regardless of whether they were the first or second to mate with a female (Bretman et al. 2009; Garbaczewska et al. 2013). This result shows that increased reproductive success can be associated with increased sperm investment and is advantageous under instances of mate competition.

Sexual conflict

There is scope for sexual conflict to occur whenever there is sexual reproduction. As discussed above, the reproductive interests of males and females are vastly different. Therefore, conflict may occur as a result of having two parents with no genetic interest in each other's future. For instance, some genes expressed in females will be in conflict with others

expressed in males (Arnqvist and Rowe 2013), hence selection favors individuals that can extract investment from their partner (Chapman et al. 2003). Sexual conflict largely acts as a destructive process that can impose substantial costs on both sexes and arises from males and females investing in different reproductive strategies (Parker 1979).

Interlocus sexual conflict

A considerable amount of research has focused on interlocus sexual conflict, which occurs due to the interactions of alleles present at different loci in the two sexes. Conflict can occur over female mating rate, parental effort, and fertilisation (Arnqvist and Rowe 2002). This means that males may evolve a trait that causes females to mate at a higher rate, which will be advantageous to the male, but will come at a cost to females. This in turn will impose counter selection on females to regain control over her ideal mating rate, which will in turn be detrimental to males. This antagonistic interaction can lead to males having higher fitness optima at the expense of females, and can occur in converse where females have increased fitness at the expense of males (Chapman et al. 2003). An example of inter-locus sexual conflict can be seen in water-striders of the genus *Gerris* (Heteroptera: Gerridae), where males have developed grasping structures that increase male mating success, but confer costs in females in terms of increased energetic expenditure and predation risk (Arnqvist and Rowe 2002). The costs imposed on females leads them to develop anti-grasping counter adaptations, which make the male traits less effective. This pushes selection towards favouring females, at a cost to reduced male mating success. The escalating conflict of interests is

closely aligned across water-strider species, meaning that grasping adaptations are more pronounced in species that have experienced high levels of conflict in the form of adaptations and counter adaptations between the sexes. This interlocus sexual conflict can lead to diverged populations, where males are well adapted to grasping females and females are well adapted to prevent male grasping, within their respective populations.

Interlocus sexual conflict over female mating rate can also promote evolution of male traits, such as harmful genitalia (Perry and Rowe 2015) that are used by males to force females to mate at a high rate, or toxic ejaculates which stimulate the female to lay more eggs than is advantageous for her and physically damage female reproductive tracts, which may also significantly reduce her lifespan. (Wigby and Chapman 2005; Hotzy and Arnqvist 2009). These harmful male traits are likely an evolved response to sperm competition. For instance, in seed beetles, where males' intermittent organs damage the females' reproductive tract as a result of males hanging on during copulation to prevent from being displaced by a rival male. This in turn leads to counter adaptations with females developing thicker reproductive tracts to prevent damage (Rönn et al. 2007), as females would be expected to oppose harmful male adaptations with counter-adaptations. Therefore, interlocus sexual conflict can also lead to lower reproductive success, shorter lifespan and faster ageing in one or both sexes. In general, interlocus sexual conflict can lead to adaptations in either sex that bias the outcome towards their own reproductive interests and fuel co-evolutionary arms races between the sexes, where adaptations in one sex can be harmful to the other and vice versa (Parker 1979).

Selfish genetic elements

Conflicts within the genome do not just stem from competition between sexually antagonistic alleles. Genomes are also vulnerable to Selfish Genetic Elements (SGEs), which subvert the usual patterns of Mendelian inheritance. While most genes in a sexually reproducing organism are transmitted to 50% of offspring, SGEs can be inherited by up to 100% of resulting progeny (Burt and Trivers 2006; Dyson and Hurst 2004). This disruption of the usual patterns of DNA replication by SGEs often results in fitness costs to the rest of the genome (Burt and Trivers 2006). SGEs are ubiquitous in living organisms and have had major impacts on the evolution of sex and genetic systems (Hurst and Werren 2001; Burt and Trivers 2006). The manipulation by SGEs of a host and the host's reaction to the manipulation is suggested to be important in the evolution of sexual reproduction and in shaping mating systems. SGEs manipulate host reproduction and gametogenesis in a variety of ways to augment their transmission. For example, many SGEs are only transmitted from mother to offspring and have evolved a variety of strategies to increase their transmission, involving male-killing and feminisation of genetic males. For example, the butterfly *Danaus chrysippus* is host to a maternally inherited male-killing bacterium. These bacteria spread when male death benefits their female siblings, who aid the bacteria's transmission (Jiggins et al. 2000). Such manipulation may result in a female-biased population sex ratio, whereby males get scarcer as the frequency of sex-ratio distorting SGEs increases. For example, in the fruit fly *Drosophila*

pseudoobscura sex ratio distorting genes can lead to >95% female biased broods (Beckenbach 1996). Sex-ratio distortion will, in turn, affect sexual selection where the rarer sex should have the greatest reproductive success. Therefore, producing females rather than males in female-biased populations is costly for host nuclear genes (Charlat and Hurst 2003). *Wolbachia* are common, maternally inherited bacteria, present in most arthropod species (Werren et al. 1995). *Wolbachia* has been shown to further its transmission by killing the sons of infected females in *Lepidoptera* (Jiggins et al. 2000) and feminising genetic males in some species of *Lepidoptera* (*O. furnacalis*), where infected females produced twice as many daughters as uninfected ones (Kageyama et al. 2002). Therefore, the strength of selection for resistance genes heavily depends on the frequency of the *Wolbachia* infection. Thus, the more biased the population sex ratio, the stronger the cost of sex-ratio distortion. Clearly SGEs can have dramatic impacts on the reproductive biology of insects and, consequently, sex ratio distorters have scope to affect entire mating systems.

Sex chromosome meiotic drivers are another type of sex-ratio distorting SGE; they influence the transmission of X and Y chromosomes from individuals of the heterogametic sex (Jaenike 2001). Typically individuals produce offspring at a sex ratio of roughly 50:50. However, in some species sex-linked meiotic drivers can create highly sex biased broods, potentially causing population level extinction due to the lack of individuals of one sex (Price et al. 2010; Pinzone and Dyer 2013). Meiotic drivers are common in nature, having been found so far in insects, mammals, angiosperm, and recently in birds (Jaenike

2001; Knief et al. 2015). In males, meiotic drivers increase their transmission by causing the failure of sperm that do not carry the driver (Price and Wedell 2008). This is due to meiotic drivers frequently targeting spermatogenesis through killing of non-driving sperm, resulting in reduced sperm number of male carriers (Price et al. 2014). This transmission advantage means that drive-bearing chromosomes should spread rapidly to fixation. However, meiotic drivers are often found at stable frequencies in natural populations (Dobzhansky 1958; Dyer 2012). What maintains the stable co-existence between driving and non-driving chromosomes remains unclear.

A potential explanation for why meiotic drivers do not reach fixation is that they impose costs on their hosts. Meiotic drivers are known to reduce the competitive ability of male carriers. For example, in *Drosophila pseudoobscura* drive-carrying males are frequently disadvantaged in sperm competition compared to non-drive males. Drive males have a severely reduced number of sperm compared to non-drive males, and as a consequence have reduced sperm competitive ability (Price et al. 2008). Therefore, the proportion of offspring sired by drive males is greatly reduced in the presence of a non-drive male (Wilkinson and Fry 2001; Price et al. 2010; Manser et al. 2011). Equally, meiotic drivers may also impose direct costs to female reproductive output. For example, reduction in fecundity compared to non-meiotic drive females has been reported in *D. pseudoobscura* females (Wallace 1948, Edwards 1961, Beckenbach 1983). However, in general, the literature surrounding the cost to females carrying meiotic drivers remains relatively unexplored, with results so far proving

inconclusive. It has yet to be demonstrated that costs to drive-carrying females are able to regulate the frequency of meiotic drivers in natural populations. The importance of fecundity costs to female carriers for regulating the frequency of meiotic drive has recently been examined in a model by Holman et al. (2015). The criteria put forward by this model are examined in chapter three.

Scope of the thesis

In this thesis I am interested in the role of conflict and competition in shaping mating systems. Specifically, I will be focusing on two aspects that are integral to determining reproductive fitness, namely male-male competition and conflict that stems from selfish genetic elements that distort the sex ratio.

The role of male-male mating competition in moths

This thesis addresses questions about what determines variation in male reproductive success, focusing explicitly on variation in male mating success. Firstly, I explore what the variation in mating success is in a competitive scenario and what traits make a male competitively successful, with regards to key life history traits such as body size, development time and longevity. This question is addressed using the Indian meal moth (*Plodia interpunctella*). Previous assays have explored the evolved changes in life-history traits (body size, development time and longevity) and associated fitness consequences between diverged populations evolving under different opportunities for sexual selection and sexual conflict, whilst also being exposed to male biased mating competition (a caveat being that previous assays were performed in the absence of any mating competition). Sexual conflict faced by the sexes is also

likely to differ in male biased populations, due to increased male-male competition. Since male-male competition is higher in the male biased populations, I have decided to use moths that have evolved under a 3:1 sex ratio bias. I expect life history traits that may be related to male competitive ability, such as body size, longevity and development time, to be more exaggerated in these moths. Therefore, moths used in this experiment were mated competitively at 3:1 sex ratio's, reflecting the sex ratio under which they have evolved and competed, to give a more realistic measure of male mating success. I expect to find similar patterns to previous studies on *Plodia interpunctella*, which show that shorter development and longer lifespan leads to higher mating success, with body size having little to no effect.

The role of sex ratio distorters: cost to female flies

I also investigated the importance of potential costs to carrying a meiotic driving sex ratio distorter (SR^x), present on the X chromosome in female *Drosophila pseudoobscura*. To date, research has been predominately focused on cost to males and is largely overlooked in females, with the available data being highly contradictory. Here I aim to quantify the cost of carrying driving SR^x chromosomes, for both heterozygote and homozygote females, by comparing the lifetime fecundity of females carrying 0, 1 or 2 copies of SR^x . These results will help to determine if there are sufficient fitness costs to homozygous females to stabilise the frequency of SR^x in natural populations, as predicted by theory (Holman et al 2015). In conjunction with Holman's predictions, I expect that homozygote females

suffer severe fecundity costs compared with heterozygote and normal females and my results should corroborate Holman's model, which tries to explain how SR is regulated in natural populations of *Drosophila pseudoobscura*.

Chapter 2: Variance in three life history traits?

Abstract

Male-male competition can lead to sexually selected traits that aid mate acquisition. Males' mating success will largely determine their overall reproductive success and may be related to specific life-history traits, which may reflect males' genetic quality. Here I used replicate populations of the Indian meal moth (*Plodia interpunctella*) that have been evolving under male-biased adult sex ratios for >100 generations. Moths were mated competitively at ratios of 3:1 and 12:4 males to females, to quantify variation in male mating success. Three life-history traits with the potential to be related to male mating success were also measured (development time, longevity and body size), in order to determine their relationships to male mating success, under a competitive scenario. There was evidence of large variation in mating success in *P. interpunctella* despite having evolved under intense mating competition for many generations. I discuss reasons for the maintenance of this variation and the role that key life-history traits may play in influencing male mating success.

Introduction

Darwin (1871) defined sexual selection as the advantage that individuals have over other individuals of the same sex, in regards to sexual reproduction. This is most pronounced through intra-specific competition between males for access to females. One consequence of intra-specific competition is that sexual selection can frequently drive trait values beyond their naturally selected optima (Hosken and House 2011). This is most evident through male-male competition where males compete for a limited resource of females. Males may develop traits that aid them in mate acquisition. For example, in insects, such as Rhinoceros beetles, larger body size, higher aggression and large appendages all contribute to increased male reproductive success (Lavine et al. 2015). In addition, female preferences may also drive increased male trait values, which will allow them to appear more attractive to females (Anderson 1994). The variance in the number of mates within sexes will determine the strength of sexual selection and allows us to predict how the sexes approach an opportunity to mate (Borgia 1979). For example, the sex with the greater variance in mating success may have more pronounced features than unsuccessful individuals; such as brighter colouration in male *poeciliid* fishes, which is preferred by females (Endler 1984) and larger antler size in male red deer, increasing their fighting success (Clutton-Brock and Guinness 1982). Therefore, a combination of traits that enhances a male's competitive ability will contribute to increased variance in male mating success and therefore determine the strength of sexual selection in males.

Measuring variation in male mating success (the total number of females a male mates with) is a good way to determine a male's reproductive success. A study in the fruit fly *Drosophila melanogaster* confirmed that variance in male reproductive success is >15 times greater than in females and largely due to variation in mating success (Pischedda 2010). Therefore, the opportunity for pre-mating sexual selection to occur in male fruit flies is strong. A follow up study, exploring the relative importance of pre- and post-copulatory components of reproductive success, showed that post-copulatory processes, such as sperm competitive success, can also be an important component of overall male fitness (Pischedda and Rice 2012). However, pre-copulatory sexual selection, in terms of variation in male mating success, was found to be the strongest determinant of a male's lifetime reproductive success. This is because successful mating is a prerequisite for post-copulatory sexual selection (Hosken and House 2011).

Male reproductive fitness is governed by traits that influence the number of females inseminated and by traits that increase males' ability to fertilise eggs in competitive scenarios (Pizzari and Birkhead 2002). For example, red jungle fowl (*Gallus gallus*) live in social groups where dominant males gain the majority of matings. The rank that each bird has within the social group also influences the outcome of competition over access to females and is therefore targeted by intra-sexual selection. The mating success of each bird can be determined by the expression of phenotypic traits, such as comb size and particularly comparative changes in comb size, which covaries with the social status and condition of the bird (Parker et al. 2002). Therefore, dominant males will achieve higher reproductive success than subordinate males by

having access to more females. Recent reports suggest that in red jungle fowl groups, as females become more polyandrous, mating success becomes comparably high for all males. This increases average mating success whilst simultaneously reducing the variance that may ultimately lead to reduced pre-copulatory sexual selection (Collet et al. 2012).

Selection on males to mate with females is strong, and frequently leads to male-male competition for the most females and/or the best quality females. Male-male competition drives the evolution of traits that increase competitive success in males. Life-history traits, such as male body size, have been shown to increase males' success in pre-mating competition in many animals (Clutton-Brock 1988), including insects. For example, in the tarantula hawk wasp (*Hemipepsis ustulata*) larger males are more adept at obtaining matings and keeping territories (Alcock 1981), and females prefer larger males (Thornhill and Alcock 1983). Although body size is an important factor in determining competitive ability in many species of males, this is not always the case (Fairbairn 1988; Cook et al. 1997). There are many other behavioural and phenotypic factors that can determine a male's competitive success.

Rapid development is another life-history trait that may also increase a male's chances of finding a mating partner. Developing and eclosing faster than your competitors increases the chances of successful copulation, especially when there is a limited number of females. Increased mating success as a result of shorter development time has been reported in many male species of insect (Nylin and Gotthard 1988). This is particularly evident in protandrous mating

systems of insects, where males emerge before females to maximise their expected number of matings with virgin females (Wiklund et al. 1992). Therefore, any males that emerge after the females will be penalised by having fewer mating opportunities. Equally, longer lifespans are advantageous to males and can increase males' mating success because it allows a longer time frame in which to secure a successful copulation, as a result of greater encounter rate. This association has been shown in moths (Lewis 2005) and damselflies (Banks and Thomson 1985). For example, where a major determinant of mating success is the number of days a male spends at a breeding site.

Mating in a competitive scenario means outcompeting your competitors. Many mating assays in previous studies were performed in the absence of any competition (Lewis et al. 2011; Lewis et al. 2013; Willis 2015). However, they have shown that in the Indian meal moth (*Plodia interpunctella*), males that are better at obtaining matings are also better at 'seducing' non-virgin females to remate. This indicates that some males appear to be inherently more appealing to females and that female preferences will be less discriminatory after a mating. Still, these conclusions were drawn in the absence of any male-male competition. To fully understand the importance of pre-mating success in *P. interpunctella*, the variation in male mating success in competitive scenarios must first be examined

In this study I determined the extent of variation in male mating success, in a competitive scenario, in the Indian meal moth and quantified traits that were potentially associated with this variation, focussing on life history traits likely to

influence male mating success: body size, development time and longevity. The aim was to examine whether life history traits differ between successful and unsuccessful males. To determine the importance of variation in the strength of male-male competition for trait evolution, I assessed male mating success in diverged moth populations that had evolved under a male biased level of sexual selection for many generations. I predict that my results will show similar patterns to previous studies, which have indicated that faster development and longer lifespan increase mating success, whilst body size has no effect on mating success. If this proved to be the case, then my study will add weight to the previous studies (Lewis et al. 2011; Lewis et al. 2013; Willis 2015) that have examined life history traits in the absence of competition, as well as providing a more realistic measure of male-male competition and mate-choice.

Moth Materials and methods

Study species

The Indian meal moth (*Plodia interpunctella*) is in the order *Lepidoptera* (butterflies and moths). The major determinant of female fitness in most *Lepidoptera* is fecundity, whereas male reproductive success is largely determined by the number of copulations obtained (Lewis et al. 2011). *P. interpunctella* is a polyandrous species with females mating on average 1.79 times over the course of their life (Cook 1999). The conditions that *P. interpunctella* are cultured and mated under in the lab is similar to natural

populations as *P. interpunctella* is a pest of stored products and lives in its food. Therefore, measurements of fitness in the lab should be fairly similar to those in natural populations. Moths used in this study have been evolving under biased adult sex ratios resulting in different strengths of sexual selection for >100 generations in three selection treatments: male biased, female biased and equal sex ratio, with each treatment having three replicates each. The moths used in this study have evolved under a male biased (MB) adult sex ratio that has altered the reproductive dynamic between males, in particular by changing the intensity of pre-mating male-male competition. Two of the replicate populations were used in this experiment (MB1 and MB3). Two replicates were used as I needed to generate large numbers for my mating assays and the replicates are not an exhaustive stock, as they are required to maintain future generations. However, the two replicates have evolved under the exact same conditions and have in the past shown very similar results (Willis 2015). In this study, I used no control lines i.e moths at a 1:1 ratio. The main question that I was interested in was what the variation in mating success was in a competitive scenario, and not how competitive males are under an equal sex ratio. This thesis is focussed on what occurs, when you alter the strength of selection to have a high level of male-male competition and at an equal sex ratio there isn't as much scope for competition. It would have been insightful to have compared the mating success of males from the male biased treatment and female biased treatments, to see what occurs when you alter the strength of selection to have high versus low levels of male-male competition. However, given the time restraints, this was not possible.

Initially an outbred stock population of *P. interpunctella* was established from individuals collected in Perth, Western Australia in 2001. The larvae were reared on a diet of bran, yeast, glycerol and honey and kept in an incubator set to $28 \pm 1^{\circ}\text{C}$ with a 16L: 8D photoperiod (Gage and Cook 1994). From this original stock population, nine experimental evolution populations were set up at different adult sex ratios that have been maintained in the laboratories at University of Exeter, Penryn Campus, Cornwall. Only adult mating competition is manipulated in this experiment, whereas the larval conditions are the same for all treatments. Here I will be explicitly focusing on MB populations. Male-biased populations were established by randomly selecting $n=120$ males and $n=40$ female moths, larvae were randomly selected from each evolving population and housed in same-sex pots until adult eclosion. Larvae can be easily sexed due to males having pigmented testes, which are visible through the body cavity wall on their dorsal side (Ingleby et al., 2010). Upon eclosion, adult moths were placed in an egg collector consisting of an inverted 1 litre pot with mesh fabric across the bottom. An inverted stockpot was then placed in a funnel, which was attached to a conical flask. After a period of 72 hours of mating interactions, eggs were collected and used to establish the next generation of the evolving population. This procedure had been repeated for over 100 generations at the time of data collection. Each population was stored in the same incubator set to $28 \pm 1^{\circ}\text{C}$ with a 16L: 8D photoperiod, throughout the experiment.

Experimental populations

MB stock population moths were first mated separately at a ratio of 3:1 according to the mating treatment to generate our initial supply of moths. All of the eggs were collected in a conical flask over a three-day mating period and the date of egg laying was recorded. Eggs were then left to hatch in large stock pots of moth food. Larvae were collected at the 5th instar stage from the three MB replicate populations, but larvae were not separated according to population. At least 300 males and 100 females were placed into single-sex pots of moth food. Same-sex pots were used to ensure that all *Plodia* moths used in this study were virgins. The effect of larval over-crowding and competition was removed by rearing the larvae with an excess of food. The larvae were then left to develop into adults in an incubator set to $28 \pm 1^{\circ}\text{C}$ with a 16L: 8D photoperiod (Gage and Cook 1994).

Moth marking

For each treatment the same-sex pots were checked each day for adult eclosions. All adults were removed on the day of eclosion via electronic pooter. The date of eclosion was noted to determine the development time of each individual moth. Male moths were placed in individual vials and placed on ice. Males were mated at a ratio of 3 males to 1 female. It was later decided that, as a better measurement of variation in mating success, a higher number of males and females should also be allowed to compete and

mate together so some males were mated at a ratio of 12 males to 4 female. The reason I changed the ratio to a 12:4 from a 3:1 is because the proportion of mating in the latter is dependent on one female mating, whereas having 12 males and 4 females allows more scope for competition. Ideally I would be able to record 120 males and 40 females, however, as that was not feasible I had to compromise to a 12:4 ratio because this was as many males as I was capable of marking and monitoring, in order to accurately record their matings. All eclosing males were split into groups and each group, and individual within a group, were given a unique ID. This was achieved by randomly assigning a colour (white, red, blue) to each moth using an Edding 780 paint pen, and marking each individual on the abdomen with a small dot of paint. In the 12:4 ratio, nine of the moths had two small dots of paint, for example white and red, allowing us to give the 12 moths unique ID's. The paint on each moth was left to dry for 5-10 minutes, before placing male moths in a pot with their unique ID number. Each marked moth was then left for 24 hours before mating for any odours from the pen to evaporate as moths use pheromones to find mates. This procedure ensured that each moth survived the marking procedure and that the smell of paint dissipated.

Mating assays

After 24 hours post-marking, virgin females that were no more than three days old were introduced to the males. All vials were monitored for four hours,

during which time I recorded each individual that gained copulation, by determining the colour ID on the males abdomen. A mating was quantified by attachment for more than 30 seconds. After four hours, the females were removed, placed in an individual Eppendorf tube labelled with their specific ID and frozen. 469 males were mated in the 3:1 ratio, in 156 trials over three consecutive days, to separate virgin females. All copulations over the three days were recorded. After the third day the males were left in the vials together and checked everyday as a measure of longevity.

The protocol for mating moths at a 12:4 ratio only differed from the 3:1 ratio in two ways. Firstly, instead of vials the moths were mated in larger 0.5 litre pots. Secondly, males and females mating to the marked individuals were the same individuals over the three-day period, but were removed each day after the four-hour observation period. This three-day mating period reflected the protocol under which the moths have evolved. A total of 696 males were mated in the 12:4 treatment in 58 trials.

Life history trait measurements

Body size was measured for each experimental moth upon death. The length of the right forewing was taken as a standard measurement, to represent body size in both sexes. In order to see the veins of the wings it was necessary to use fine forceps to remove the right forewing of each moth. The wings were then immersed in a solution of 90% alcohol, followed by 10%

hydrochloric acid solution and then bleach (Reid, 1976). The wings were finally washed in distilled water, mounted on a slide and allowed to dry. The length between the vein one junction and point of wing insertion was then measured under a 'Leica dsc' microscope. Body sizes were determined using the computer imaging software 'ImageJ'. Development time was measured in terms of the number of days from oviposition to eclosion as an adult. Longevity was measured in number of days from the day of eclosion as an adult until the death of the moth.

Analysis

All analyses were conducted using R version 3.2.2 (R Core Team, 2013). Data was analyzed using mixed effect models, with gaussian error structure. All factors and interactions were included in the initial models, the fixed effect was mating success and random effects included body size, longevity, development time, replicate and group, with non-significant factors removed in a stepwise method to produce a final model.

Results

Mating success 3:1 ratio

Out of the 469 males (across 156 groups) that had the opportunity to mate over a three-day period, 198 (42%) males gained successful copulations. Out of those 198 males, 78 (16%) mated multiply over the three-day period and 29 (6.2%) of those 78 gained successful copulations on each of the three days.

Mating success 12:4 ratio

In a more competitive scenario, out of the 696 males (across 58 groups) that had the opportunity to mate over three-day period, 229 (34%) males gained successful copulations. Out of those 229 males, 96 (14%) mated multiply and 21 (3%) of the 96 males gained successful copulation on each of the three days.

Life history traits

My aim was to explore what traits are associated with high and low mating success under male-male competition. Key life history traits were measured: body size, development time and longevity. In the 3:1 ratio it was found that males that gained copulations had, on average, significantly shorter

development time (GLM: $F_{1,468} = 22.76$, $P = <0.001$) and longer lifespan than unsuccessful males (GLM: $F_{1,439} = 19.5$, $P = <0.001$), whereas there was no significant difference in body size (GLM: $F_{1,414} = 1.2$, $P = >0.05$).

In the 12:4 ratio I found that males who gained copulations had significantly shorter development times than unsuccessful males. (GLM: $F_{1,669} = 8.14$, $P = 0.005$). There was also a marginally non-significant difference between successful and unsuccessful males in longevity (GLM: $F_{1,626} = 3.35$, $P = >0.05$) but no difference in body size (GLM: $F_{1,588} = 0.39$, $P = >0.05$). To take into account the fact that we had a total of 58 groups, the group number was added as a random effect in order to ascertain if life history traits within each of the 12:4 groups differed significantly. Out of the total 58 groups, there were no significant differences between any of the life history traits (GLM: $F_{1,560} = 0.83$, $P = >0.05$).

I wanted to examine what maintains variation in male mating success. One possibility is that there are trade-offs between traits known to be associated with high mating success (i.e. the three life history traits). The relationship between these three life history traits was examined using a Spearman's rank correlation. I found a negative relationship between body size and development time ($r_s = -0.34$, $n=588$, $p < 0.001$), a positive relationship between body size and longevity ($r_s = 0.16$, $n=574$, $p < 0.001$), and a negative relationship between development time and longevity ($r_s = -0.15$, $n=626$, $p < 0.001$) (2.2).

Life history traits of mated males

I examined using post-hoc tests whether more successful males differed in their life history traits depending on whether they mated once, twice or three times using figure 2.1). Males that gained three copulations had, on average, significantly smaller body size (GLM: $F_{1,133} = 8.8$, $P = 0.003$), no difference in lifespan (GLM: $F_{1,143} = 2.5$, $P = >0.05$), and longer development time (GLM: $F_{1,151} = 5.8$, $P = 0.017$) than males that mated once.

When comparing males that mated three times to males that mated twice I found, on average, no significant difference in body size (GLM: $F_{1,87} = 1.81$, $P = >0.05$). However, males that mated three times had significantly shorter lifespans (GLM: $F_{1,92} = 5.35$, $P = 0.02$) and longer development times (GLM: $F_{1,94} = 15$, $P = 0.004$).

Males that mated once and twice were also compared, with males that mated once having significantly larger body size (GLM: $F_{1,184} = 4.25$, $P = 0.04$), and with no significant differences in longevity (GLM: $F_{1,197} = 0.29$, $P = >0.05$) or development time (GLM: $F_{1,205} = 0.5$, $P = >0.05$).

The reason that I did not quantify whether the differences in proportion of males mating differs significantly between the 3:1 and 12:4, was because I am not comparing like with like. The 3:1 and 12:4 ratios differ in the fact that the 3:1 only have one female and if she doesn't want to mate then this has a

much higher impact on mating success than in the 12:4 ratio in which there are four females to mate with.

Figure 2.1: The average (± 1 SE) body size, longevity and development time of unsuccessful males and males that gained either one, two or three matings over the three-day period (12:4 treatment).

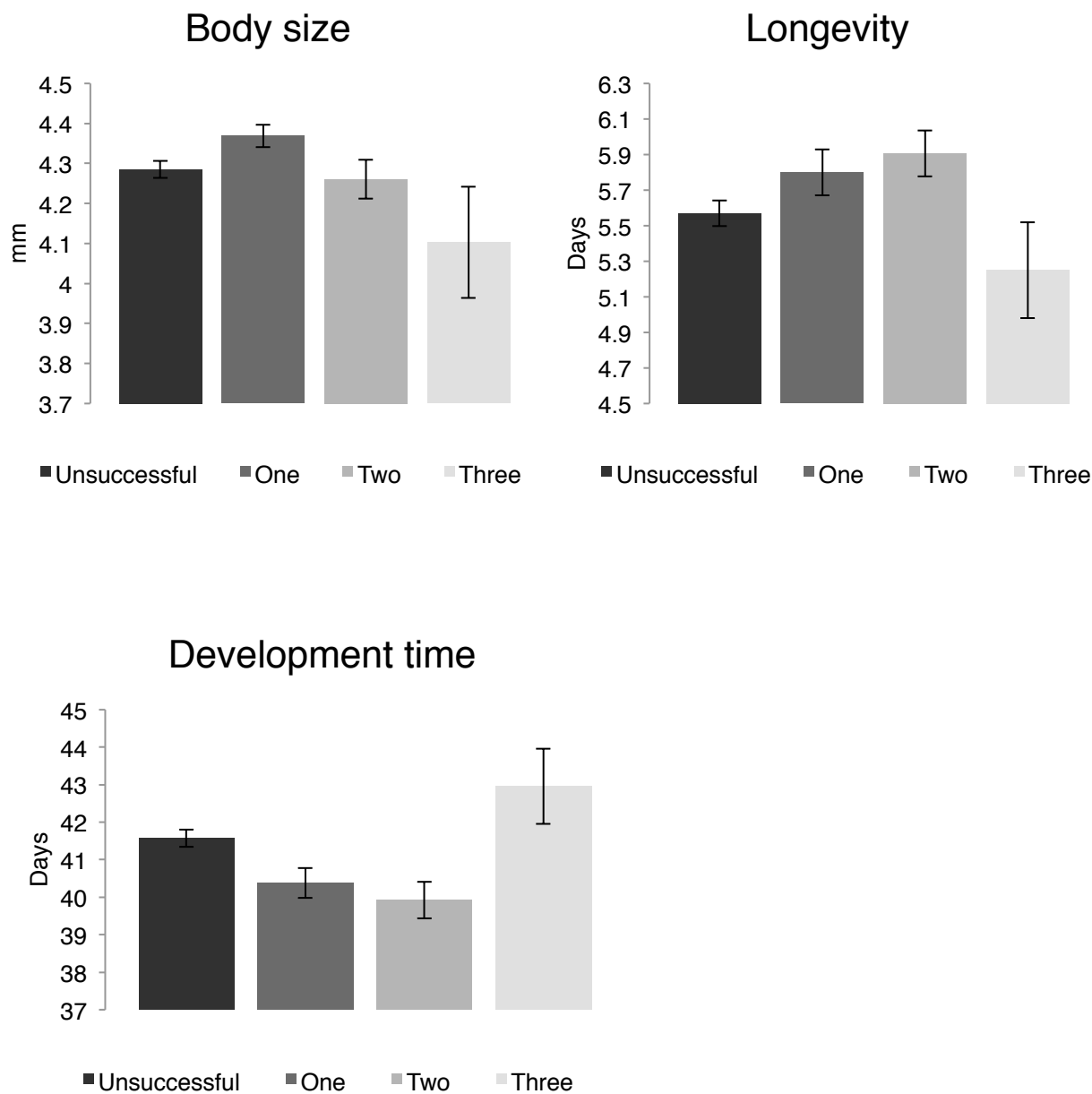
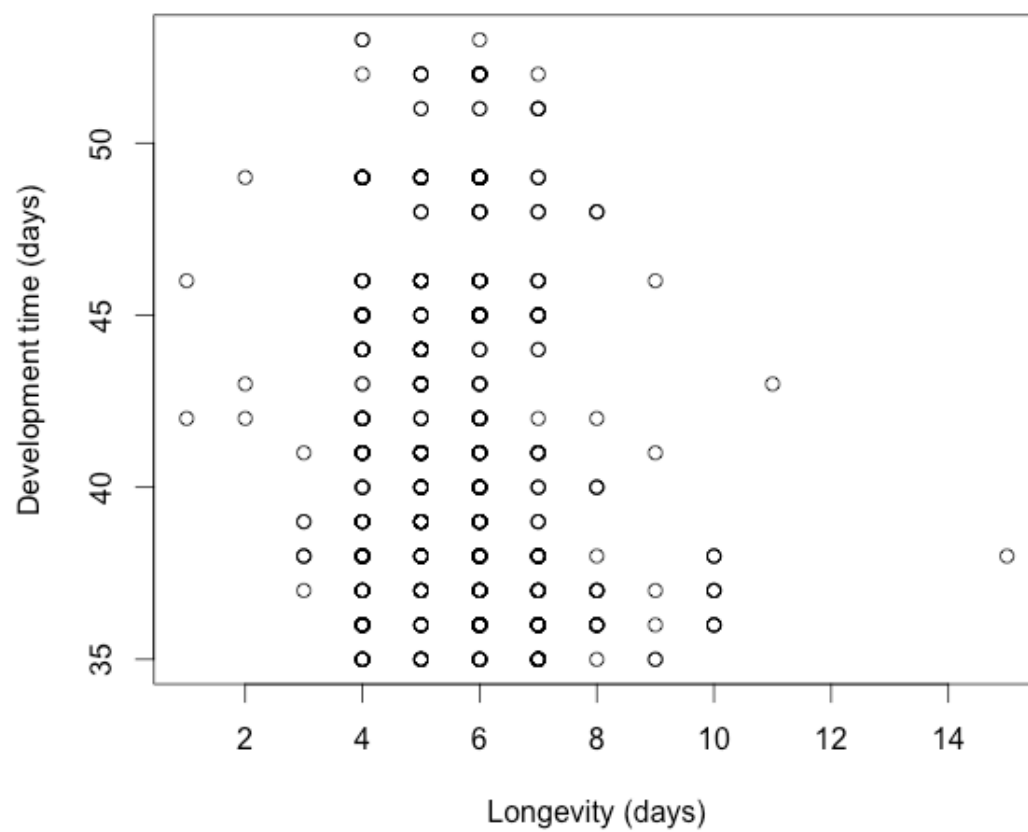


Figure 2.2: The negative correlation between development time and longevity in the 12:4 male moths. R^2 value -0.15



Discussion

Here I have demonstrated that, under competitive scenarios, there is large variation in male mating success in *P. interpunctella*. Between 34-42% of males gained at least one copulation over the three-day period. Male re-mating frequency was found to be fairly consistent in both treatments, at around 15% (39-42% of successful males remated). Males' reproductive success is largely determined by the number of matings obtained and is limited by males' ability to compete over or attract females (Wade 1979). Variation among males in mate number therefore reflects variation in male-male competitive ability, as well as variation in male attractiveness to females. Large variation in mating success means that there is a strong opportunity for pre-mating sexual selection to occur in males (Clutton-brock 1988). The moths in this experiment have been evolving under a male biased adult sex ratio for >100 generations, resulting in high male-male competition. Despite this long history of male-male competition there is still substantial variation in male mating success, suggesting that mating success is non-random.

To determine what traits are associated with variation in male mating success under a competitive scenario, I focussed on life history traits known to influence male mating success. Previous work in *P. interpunctella* has shown that male mating success is associated with rapid development time and longer lifespan (but is not influenced by a difference in body size) when mating under an equal (Lewis et al. 2011) or male biased sex ratio (Willis 2015). Altering the adult sex ratio to be male biased increases the intensity of

male-male competition for access to the limited resource of females (Wigby and Chapman 2004). The moths used in this experiment had evolved under male biased adult sex ratios for over one hundred generations at the point of mating and hence have been subject to sustained high levels of male-male competition. I found that successful males, on average, tend to develop quicker, live longer, and were similar in body size in both treatments. Within each of the 12:4 groups I also found a trend towards successful males being longer-lived and having shorter development times, compared to the average for each group. Therefore, the observed differences in life history traits between males that gained matings compared to unsuccessful males is consistent with previous findings (Lewis et al 2011).

This experiment examined two groups of males. Unsuccessful males, who never got to pass on their genes, and successful males, whose heritable traits were passed on to the next generation. A male's reproductive fitness is governed by traits that influence the number of females inseminated and by traits that increase males' ability to fertilise eggs in competitive scenarios (Pizzari and Birkhead 2002). In many organisms pre-copulatory mechanisms favour large body size, such as male-male competition and female mate choice (Andersson and Simmons 2006). However, our results in *P. interpunctella*, suggest that successful males are no larger than unsuccessful males, verifying previous findings which demonstrated no strong relationship between male size and mating success in this species (Cook 1997 et al.; Willis 2015).

Assaying the moths over a three-day period reflects the conditions under which they have evolved for over one hundred generations. In nature, protandrous mating systems are common amongst insects living in seasonal environments with non-overlapping generations (Bulmer 1983). It is not clear whether natural populations of *Plodia interpunctella* are always non-overlapping, because they are grain store pests and more than one breeding generation may be present at any one time. Developing and eclosing faster than their competitors will increase males' chances of gaining successful copulations (Morby and Ydenberg 2001). However, in this study there is no selection for rapid development time in relation to mating opportunity as I decided when the males got to mate, and consequently there is no advantage to a male being early. Sexual dimorphism is apparent in *P. interpunctella* with males developing faster than females (Willis 2015). This may reflect the need for females to develop for longer in order to accumulate enough nutrients for egg production, as they do not feed as adults (Marshall 1982). However, it is also possible that rapid development time is advantageous to males, as faster developing males have higher mating success. Perhaps developing quickly reflects overall genetic quality, with male attractiveness and offspring development time being genetically correlated as is the case in some species of beetles (Moore 1994). High levels of larval competition have been shown to reduce adult lifespan in *P. interpunctella* (Gage 1995), as increased competition reduces a male's ability to acquire resources efficiently during larval growth. However, all moths used in this experiment were cultured with the effects of larval overcrowding controlled for. Living longer than their competitor ensures that males have a greater female encounter rate and

therefore allows a longer time frame in which to secure a successful copulation (Gage 1995). However, here only three days of mating were allowed, hence male moths do not benefit reproductively from living longer as this does not result in more opportunity for mating. Despite this, I found that males that gained copulations lived for longer than unsuccessful males. Although, males that gained the maximum of three copulations had significantly shorter lifespans than males that mated once or twice. This result may indicate a cost of mating in males, particularly in the production of sperm, which has been shown to be energetically costly (Dewsbury 1982). It is, however, unlikely that the costs of sperm production in one additional mating contributes to such a significant drop in lifespan.

So what maintains variation in male mating success and these life history traits? One possibility is that there are trade-offs between traits known to be associated with high mating success (i.e. lifespan and development time). However, there is no apparent trade-off between rapid development time and lifespan as these are both correlated with longer lived males developing more quickly. Interestingly, there are differences in life-history traits among the males that mated at different frequencies. Males that gained three copulations had shorter lifespans and longer development times compared to males obtaining two matings and longer development time, with a trend towards shorter lifespan and significantly smaller body size when compared to males obtaining one mating. Little difference in development time and longevity was found between males that mated once and twice, with body size being slightly larger in males that mated once (figures 2.1). These results contrast with previous findings that suggested that successful males develop faster and live

longer (Lewis et al 2011; Willis 2015) and is the same pattern found here for males mating once or twice compared to unsuccessful males. (NB. See my discussion, above, as to why I did not use baseline controls, as it's not entirely clear whether they are a control in regards to male-male competition). It is possible that the difference in development time and longevity between males that gain three copulations, compared to those that gain one or two, is sufficient to maintain variation in these traits, which are in turn associated with mating success. However, some caution may be warranted as the sample size is on the small side with only 21 males (3%) mating three times. Assuming this result is reliable the opposing life-history traits of successful males may maintain variation in male mating success in these moth populations.

This study only examined the effect of pre-mating success in a competitive scenario, however it is possible that the levels of sperm competition post-mating may also affect a male's reproductive success. Increased levels of male-male competition are known to affect male sperm investment in *P. interpunctella*, with moths evolving in male biased populations producing significantly more sperm than males evolving under an equal sex ratio (Ingleby et al. 2010). One possibility is that there may be trade-offs between pre- and post-mating success. Trade-offs between ejaculate components or between total sperm production and male mating success was not examined in Ingleby et al. (2010). However, trade-offs between somatic development and reproduction have been explored by Lewis et al. (2011), who found that males reared in resource-limited conditions mated less frequently than males in high quality larval conditions. However, resource-limited males allocated

adequate resources to the matings they did achieve, which ensured fertilisation success under sperm competition. The effect of trade-offs between pre- and post-copulatory sexual selection rarely act in isolation (Pischedda and Rice 2012). Therefore, future work may want to investigate post-copulatory mechanisms as well as pre-copulatory, as demonstrated by Lewis et al. (2013), who found that males that were better at obtaining matings were also better at seducing non-virgin females. Equally, sperm production and transfer was shown to be a key component of reproductive success in *P. interpunctella*, with males that produced greater numbers of sperm gaining greater paternity success. Here, I have shown clear indication that there is large variation in mating success in *P. Interpunctella*. However, it would be interesting to determine whether sperm production differs in successful moths and if this correlates with greater overall mating success in the moths used in this study.

In conclusion, this study has shown that there is large variation in male mating success both at a 3:1 ratio and a more competitive ratio of 12:4 males to females. These results corroborate previous work suggesting shorter development time and increased lifespan are associated with higher mating success in males. However, the life history traits of males that are most successful also differ from males that never got to mate and males that mated once or twice. I suggest that the differences in life-history traits between very successful and moderately successful males may be sufficient to maintain variation in mating success in *P. interpunctella*, even when evolving under intense male-male competition. I will also suggest that all future studies examining pre-copulatory mating success in *P. interpunctella*, should conduct

mating assays in a competitive environment. My results demonstrate that this method provides a truer measure of a male's ability to gain copulations and also provides clearer indications of what makes one male successful over another.

Chapter 3: The role of sex ratio distorters: cost to female flies

Abstract

Selfish genetic elements (SGE's) can have severe deleterious effects to those who carry them. Sex chromosome meiotic drivers are one such SGE that have been studied in the fruit fly (*Drosophila pseudoobscura*) for more than 80 years. Here, I examine the effects of a sex ratio distorter (SR^x). Whilst the effect of SR^x on male *D. pseudoobscura* fitness is well documented, we have little knowledge about the potential costs to females of carrying the SR^x distorter. In this chapter, the fecundity costs to heterozygote and homozygote females carrying a meiotic driving sex ratio distorter (SR^x) were explored. I discovered that females carrying two copies of SR^x suffered substantial fecundity costs across multiple genetic backgrounds and I discuss whether there are sufficient fitness costs to homozygous females to stabilise the frequency of SR^x in natural populations, as predicted by a recent model.

Introduction

Selfish genetic elements (SGEs) subvert the usual patterns of Mendelian inheritance. While most genes in a sexually reproducing organism are transmitted to 50% of offspring, SGEs can be inherited by up to 100% of resulting progeny (Burt and Trivers 2006; Dyson and Hurst 2004). This disruption of the usual patterns of DNA replication by SGEs often results in fitness costs in the rest of the genome (Burt and Trivers 2006). SGEs are ubiquitous in living organisms, although they are more prevalent in some organisms than others, they can have major impacts on the evolution of sex and bear severe genetic effects to hosts (Hurst and Werren 2001; Burt and Trivers 2006). Sex chromosome meiotic drivers are one such SGE; they influence the transmission of X and Y chromosomes from individuals of the heterogametic sex (Jaenike 2001). Typically individuals produce offspring at a sex ratio of roughly 50:50. However, in some species meiotic drivers can create highly sex biased broods, potentially causing population or even species-level extinction due to the lack of individuals of one sex (Price et al. 2010; Pinzone and Dyer 2013).

Meiotic drivers are common in nature, having been found so far in insects, mammals, angiosperms and recently in birds (Jaenike 2001; Knief et al. 2015). In males, meiotic drivers increase their transmission by causing the failure of sperm that is not carrying the driver. This is due to meiotic drivers frequently targeting spermatogenesis by killing non-driving sperm which results in reduced sperm number of male carriers. Meiotic drivers are

segregation distorters that increase their representation at the gametic level compared to non-drive bearing individuals (Burt and Trivers 2006). This transmission advantage means that drive-bearing chromosomes should spread rapidly to fixation. However, meiotic drivers are often found at stable frequencies in natural populations (Dobzhansky 1958; Dyer 2012). What maintains the stable co-existence between driving and non-driving chromosomes remains a mystery.

A potential explanation for why meiotic drivers do not reach fixation is that they impose costs on their hosts. Meiotic drivers are known to reduce the competitive ability of male carriers. For example, drive males are frequently disadvantaged in sperm competition compared to non-drive males, due to reduced fertility. Consequently the proportion of offspring sired by drive males is greatly reduced in the presence of a non-drive male (Wilkinson and Fry 2001; Price et al. 2010; Manser et al. 2011). Equally, meiotic drivers may also impose direct costs to female reproductive output, for example reductions in fecundity compared to non-meiotic drive females (Wallace 1948; Beckenbach 1983).

Natural populations of the fruit fly *Drosophila pseudoobscura* harbour “sex-ratio” (hereafter referred to as “SR^x”), a meiotic driving X chromosome that kills the Y chromosome-bearing sperm of male carriers during spermatogenesis (Beckenbach 1981; Jaenike 2001). Flies that carry non-driving X chromosomes are commonly referred to as “standard” (“ST”) flies. SR^x can result in female biased broods, as females mating with SR^x males will only sire daughters. SR^x is inherited by the offspring of females that mate with

male carriers. Consequently, for female carriers of SR^x >95% of their offspring are female (Beckenbach 1996). This gives SR^x a large transmission advantage compared to the Y and non-driving X chromosomes.

SR^x has been studied in *D. pseudoobscura* for more than 80 years (Sturtevant and Dobzhansky 1936). SR^x reduces the number of sperm male carriers produce, and as a consequence, they have reduced sperm competitive ability (Price et al 2008). Therefore, sperm competition between SR^x and non- SR^x males could prevent the spread of SR^x , due to the high likelihood of non- SR^x males gaining high paternity in competition as they produce more sperm (Taylor and Jaenike 2003; Price et al. 2010). Female multiple mating may thus undermine the transmission advantage of SR^x by promoting sperm competition. This suggestion is supported by the findings of a latitudinal cline in the frequency of SR^x , which is associated with the degree of polyandry across the USA. In northern populations SR^x frequency is low and females have high re-mating frequencies, both in the field and the lab. However, in southern populations SR^x frequency is high and females have a low remating frequency (Price et al. 2014). Experimental work has confirmed that females mating more than once can prevent the spread of SR^x through laboratory populations, whereas when females only mate once SR^x rapidly spreads, causing population extinction due to lack of males (Price et al. 2010). Conversely, SR^x frequency is higher in monandrous populations in the wild (Price et al 2014). This indicates that high frequency of polyandry, resulting in high level of sperm competition, may be effective in regulating the frequency of SR^x in natural populations.

The importance of costs to SR^x carrying females and the potential for regulating the frequency of meiotic drivers such as SR^x was recently examined in a model by Holman et al. (2015). The model shows that polyandry alone is not sufficient to prevent the fixation of X-linked meiotic drivers. This is partly due to polyandry not evolving rapidly enough to reach a high enough frequency where it can prevent the spread of SR^x . The model concludes that substantial fitness costs to homozygous SR/SR females are necessary to prevent drivers from spreading to fixation. However, polyandry, coupled with the cost to homozygous females, is predicted to effectively regulate the frequency of meiotic drivers such as SR^x in natural populations (Holman et al. 2015).

Whilst the effect of SR^x on male *D. pseudoobscura* fitness is well documented, we have little knowledge about the potential costs to females of carrying the SR^x distorter. It is possible that carrying an SR^x X-chromosome may be associated with fitness costs to females. SR^x in *D. pseudoobscura* are characterised by a set of three inversions (Wallace 1948), resulting in reduced recombination and background selection which allow the build-up of deleterious alleles (Curtis and Feldman 1980). The effective population size of SR^x in natural populations of *D. pseudoobscura* is usually relatively small (Dobzhansky and Epling 1944). SR^x can be present in up to 30% of the population, however is typically maintained at far lower frequencies (Price et al. 2014). Therefore, the small population size of homozygous SR/SR females suggests that recessive deleterious alleles are particularly likely to be present in these females. Previous researchers have investigated the costs of SR^x chromosomes to female *D. pseudoobscura*. Overall, the results from studies

examining the fecundity effects of SR^x carrying females are inconsistent (Powell 1997), with no clear pattern of any fecundity costs.

Here I aim to quantify the cost of carrying driving SR^x chromosomes for both heterozygote and homozygote females by comparing the fecundity of females carrying 0, 1 or 2 copies of SR^x . This will determine whether there are sufficient fitness costs to homozygous females to stabilise the frequency of SR^x , as predicted by the Holman et al. (2015) model. To avoid the risk that the measurements of relative fitness of SR^x were influenced by the fitness of the ST X chromosomes it is being compared against, or by epistatic interactions with the genetic background, SR^x was backcrossed into four distinct ST genotypes from two populations. Two isolines were from Northern USA, where SR^x is absent: Lewiston, Montana ($35^{\circ} 05' 00''$ N, $111^{\circ} 44' 10''$ W). The other two isolines were from Southern USA: Show Low, Arizona ($34^{\circ} 15' 00''$ N, $110^{\circ} 0' 00''$ W), where SR^x naturally occurs at high frequency (~20%). The SR^x strain used was derived from the Southern population. Therefore, these four isolines should provide a good estimate of putative fitness costs of carrying SR^x and whether genetic background is an important factor affecting such potential costs to females. In conjunction with Holman's predictions, I expect that homozygote females suffer severe fecundity costs compared with heterozygote and normal females. I expect my results to corroborate with Holman's model, which tries to explain how SR is regulated in natural populations of *Drosophila pseudoobscura*.

Materials and methods

Standard isoline flies

The laboratory populations were established from wild caught *Drosophila pseudoobscura* at Show Low, Arizona (Southern USA) and Lewiston, Montana (Northern USA), between May-June 2008 (see Price et al. 2014). Flies were caught using standard Banana baits (Markrow and O'Grady 2005), and males and females were isolated from each other and sent to the laboratory where they were stored at 23°C, with a 14 L:10 D cycle. They were reared in standard *Drosophila* vials (25 × 75 mm) on a medium of rolled oats, brown sugar, dried yeast, agar, nipagin, propionic acid and water (Shorrock 1972). The offspring of each wild caught female were inbred and used to create standard isofemale lines (lines of flies descended from single female). Repeated inbreeding ensures that isofemale lines quickly become homozygous at most alleles, meaning individuals within an isoline are genetically indistinguishable and are effectively clones. Isofemale lines are able to preserve high genetic diversity in laboratory, as homozygosity prevents adaptation to the environment (David et al. 2005).

SR^x establishment

The homozygous *SR/SR* flies used in this experiment were descended from a single SR^x male caught in Show Low, so there is only one SR X chromosome. The rest of their chromosomes have been outbred in a large population every

generation since August 2004 (Price et al. 2010). Upon capture, flies were kept on standard *Drosophila* food (see above). SR^x carrying males and females were identified by mating individuals and counting the resulting offspring sex ratios. Flies that produced less than 6% male offspring were assigned SR^x status (Price et al. 2008). To maintain SR^x in a population homozygous SR/SR females were mated to male SR^x carriers. Mating these SR/SR females to standard isoline ST/Y males produces SR^x males and heterozygous SR/ST females.

Backcrossing and introgression of SR^x

The aim of the experiment was to investigate potential fecundity costs to females of carrying SR^x . Therefore I generated females with 0, 1 or 2 driving X chromosomes. A strain of SR^x was isolated from Show Low flies and then introgressed into the ST population backgrounds by recurring crosses. These crosses resulted in flies that had the same outbred background as the ST isoline, but carried SR^x rather than the ST X chromosome. Homozygous SR/SR females were confirmed by genotyping using PCR and gel electrophoresis (methods and primers reported in Price et al. 2008). SR/SR females were then mated to ST/Y males from each of the four isolines. The F1 of these crosses created SR/Y carrying males. A large number of SR/SR females were required to backcross 1 or 2 driving X chromosomes into female isolines. Therefore, at each generation SR/SR females were crossed to SR^x males from their own isoline. The sex ratio of the offspring eclosing from each of these crosses should be >95% SR/SR female. If a male was found in a vial,

they were probably SR0 pseudo-males, which look superficially male but do not have aedeagus/claspers and are almost always infertile, however as a control the whole vial was removed from the experiment. Mating an *SR/SR* female to a standard isoline *ST/ST* male resulted in heterozygous *SR/ST* females with one driving X chromosome and *SR/Y* carrying males. Genetically identical *ST/ST* stock isolines were maintained by mating *ST/ST* females to *ST/Y* males. This process was performed for at least 7 generations before the fecundity assays were performed.

Mating assays and offspring counts

Experimental flies were collected within 18 hours of eclosion, to ensure they were virgin. All flies were transferred without anesthesia to ensure normal copulation behaviour (Barron 2000). All mating assays in this experiment involved flies in generations 7-9 to ensure that the SR X chromosome was fully introgressed into each of the four isolines (Wu and Beckenbach 1983). Twenty females were mated between either 7/8 or 8/9 generations of introgression. This measure was repeated in each of the three distinct female genotypes: *SR/SR*, *SR/ST* and *ST/ST*.

Virgin females carrying 0, 1 or 2 driving X chromosomes were collected and placed in new food vials. All males and females were 3-5 days old at the time of mating, at which age they are fully sexually mature (Beckenbach 1978). All females used in this experiment (*SR/SR*=148, *SR/ST*=148, *ST/ST*=158) were observed mating in a vial containing standard *Drosophila* food, for a 2-hour period following the start of the experiment (Price et al. 2008). Virgin males

were collected and kept in separate vials, as male-male interactions prior to mating have been shown to impact on mating behaviour and success in male *Drosophila* (Lize et al. 2012). All the males used were standard isoline *ST/ST* males corresponding to each of the four isolines that the SR^x chromosome had been backcrossed into. After the 2-hour mating period males were removed from the vials, and all successfully mated females were transferred to a fresh vial. Vials in which females failed to mate with the focal female were discarded from the experiment. Females were permitted to oviposit for 12 days in total and were moved onto fresh food every 3 days.

All offspring from each vial were counted as a measure of female fecundity (a measure that is commonly used, as it correlates with lifetime fecundity) (Taylor et al. 2008). To ensure that all the offspring had eclosed, 7 days were allowed between the first adult eclosion and offspring count. Offspring were counted using a 'LeicaL2' microscope and the sex ratio determined. The wings of focal females were dissected under a 'LeicaL2,' and a standard wing measurement (posterior cross vein to the distal extreme of the fourth longitudinal vein, following Gilchrist et al. 2001) was obtained. Body size was then measured using 'ImageJ'. All focal females were genotyped. DNA was extracted from each fly, amplified using PCR and then screened for both SR^x and *ST* genes. This procedure ensured that the introgression had worked. All females whose genotype was not as expected, (n=23 of 463) across all four isolines, were removed from the data analysis.

Analysis

All analyses were conducted using R version 3.2.2 (R Core Team, 2013). Data was analyzed using general linear models with quasi-poisson distribution to control for over dispersion, except for failure rate of females, which was analyzed using binomial errors. All factors and interactions were included in the initial models, including generation, body size, block and isoline with non-significant factors removed in a stepwise method to produce a final model.

Results

I found that there was a strong effect of carrying driving X chromosomes on female fecundity. Fecundity of *SR/SR* females was significantly lower compared to *SR/ST* and *ST/ST* females (figure 3.1), showing that females carrying 2 driving X chromosomes suffer a severe reduction in fecundity compared to *SR/ST* (Chi test: Deviance_{1,285} = 1843.9, $P = < 0.001$) and *ST/ST* females (Chi test: Deviance_{1,292} = 2520.9, $P = < 0.001$). However there was no significant difference in fecundity between *SR/ST* and *ST/ST* females (Chi test: Deviance_{1,296} = 53.7, $P = > 0.05$). Larger females tended to have higher fecundity (Chi test: Deviance_{1,336} = 210, $P = 0.0133$), nevertheless, fecundity was not significantly affected by an interaction between body size, the number of driving X chromosomes the female carried, or the isoline (Chi test: Deviance_{2,321} = -220.5, $P = > 0.05$). The age at which the female was mated had no effect on fecundity (Chi test: Deviance_{1,438} = 1.8, $P = > 0.05$). However, fecundity was significantly affected by an interaction between

genotype and isoline (Chi test: Deviance $_{9,428} = 522.7$, $P = 0.03$) (table 3.1). This was due to non-significant differences between *SR/ST* and *ST/ST* females across three of four the isolines.

The number of driving X chromosomes also affected the failure rate of mated females (the proportion of matings that produced no viable offspring despite successful mating), *SR/SR* females produced 0 offspring 23% of the time, *SR/ST* females 17% of the time and *ST/ST* only 3%. Females carrying 2 SR^x chromosomes had significantly higher failure rate than *SR/ST* females (Chi test: Deviance $_{1,49} = 12.4$, $P = <0.001$). Moreover, females carrying either one or two driving X chromosomes had significantly higher failure rate compared to standard females (Chi test: Deviance $_{2,54} = 38.4$, $P = <0.001$). However, the number of females that produced no offspring did not affect the general pattern of reduced fecundity of SR^x females. When all the crosses that produced no offspring were removed from the analyses *SR/SR* females still had significantly lower fecundity than *SR/ST* (Chi test: Deviance $_{1,233} = 1338.1$, $P = <0.001$) and *ST/ST* females (Chi test: Deviance $_{1,254} = 1402$, $P = <0.001$), whereas the fecundity of *SR/ST* and *ST/ST* females did not differ (Chi test: Deviance $_{1,271} = 0.07$, $P = 0.95$, figure 3.2). In light of this finding it can be concluded that *SR/SR* females produced fewer offspring on average than *ST/ST* and heterozygote females, even when taking crosses generating no offspring into account.

Finally, the sex ratio of focal females' offspring was found to be female biased in all treatments, against a null of 50:50 despite only mating to non-driving ST males: *SR/SR*: (Chi test: Deviance $_{1,2} = 82142$, $P = <0.001$), *SR/ST*: (Chi test:

Deviance $_{1,2} = 363983$, $P = <0.001$) and ST/ST (Chi test: Deviance $_{1,2} = 212374$, $P = <0.001$). The difference in sex ratio between SR/SR and SR/ST females was not significant (Chi test: Deviance $_{1,5} = 141$, $P = >0.05$). However, sex ratio was substantially more biased toward females in SR/SR (61%) and SR/ST (60%) genotypes compared to ST/ST (54%) females (Chi test: Deviance $_{1,9} = 200178$, $P = <0.001$).

Figure 3.1: The number of offspring produced by females in relation to the number of SR X chromosomes; *SR/SR*, *SR/ST*, *ST/ST*, across isolines (n=4 genotypes). *SR/SR* females produce significantly fewer offspring compared to *SR/ST* and *ST/ST* females across all four isolines.

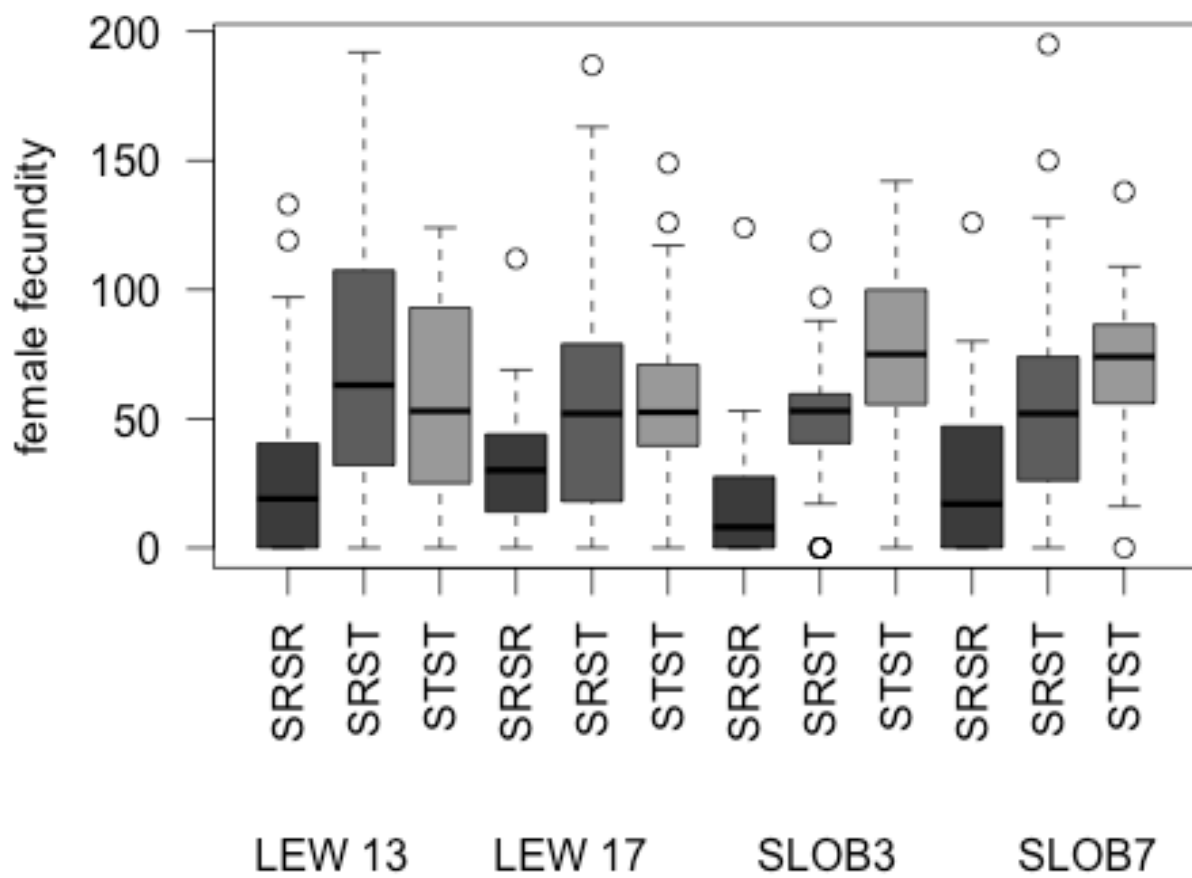
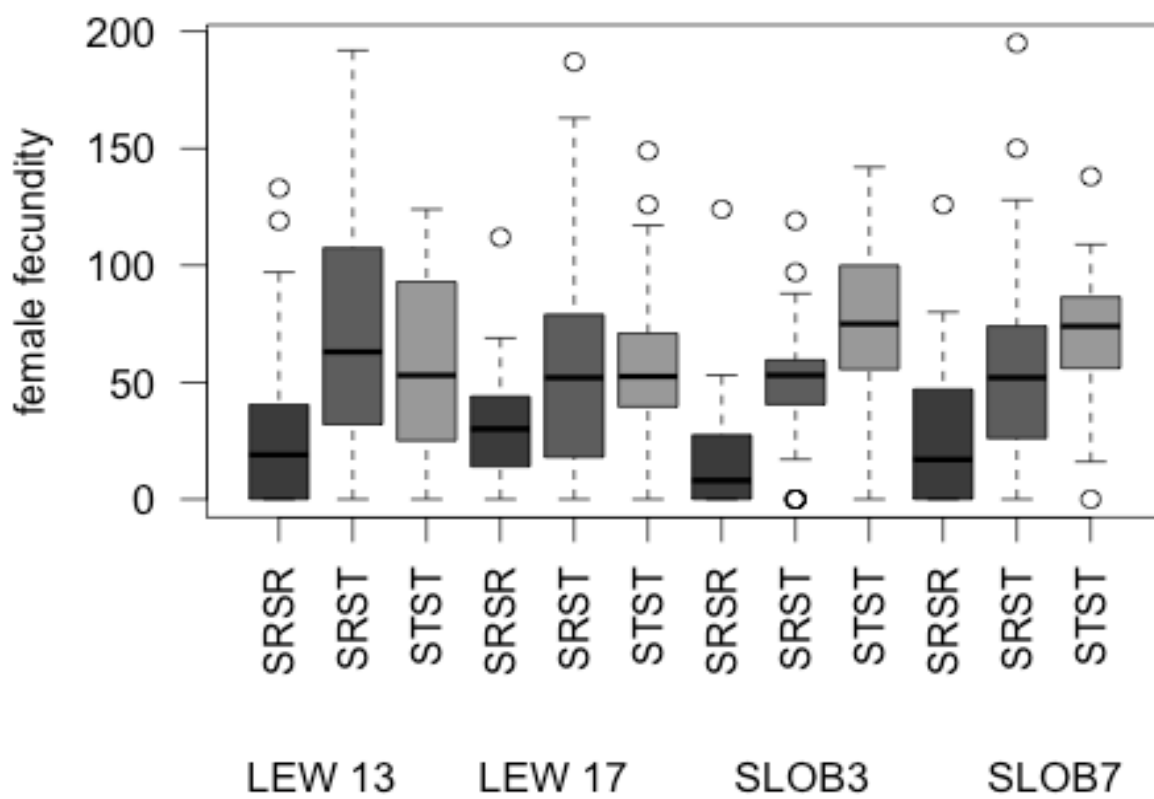


Figure 3.2: The offspring production of females in relation to genotype; *SR/SR*, *SR/ST*, *ST/ST* across isolines (n=4 genotypes), with females that produce 0 offspring removed. *SR/SR* females produce significantly fewer offspring compared to *SR/ST* and *ST/ST* females across all four isolines



Discussion

This study shows that there are substantial fecundity costs to carrying two driving X chromosomes for female *D. pseudoobscura*. Homozygous *SR/SR* females produce fewer than half the number of offspring compared to heterozygous *SR/ST* and standard *ST/ST* females. This finding is consistent across all four isoline backgrounds despite originating from two different populations. Unsurprisingly, there are some differences in the overall fecundity between the isolines. However, the impact of carrying two SR^X chromosomes resulted in consistent and drastic fecundity costs across all isolines (with no significant genotype and isoline interaction), indicating that this is a robust finding. Unexpectedly, female offspring production was female biased across all treatments when mating with an *ST/ST* standard male, with *SR/SR* (61%) and *SR/ST* (60%) females producing significantly more female-biased broods than *ST/ST* females (54%). In short this result is hard to explain as previous research has not documented sex-ratio drive through females and, as yet, it is not clear whether the observed female-bias represents a case of female drive or differential male offspring mortality. Interestingly, the number of SR^X chromosomes a female carried predicted whether she would fail to produce any offspring at all, with 23% of *SR/SR* and 17% of *SR/ST* females failing to produce offspring despite being observed to successfully copulate. This is a remarkably high proportion in comparison to *ST/ST* females, who only failed to produce offspring 3% of the time. However, even when excluding females with zero offspring production from the analysis, *SR/SR* females still showed a significant reduction in fecundity, suggesting there may be serious sterility costs to females that carry SR^X .

The results from previous studies focusing on fecundity costs to females carrying SR^x are inconsistent. Wallace (1948), Edwards (1961) and Beckenbach (1983) reported that homozygous SR/SR females had substantially reduced fecundity compared to heterozygous SR/ST females and non-carrying ST females, although the reliability of Beckenbach's (1983) results are questionable due to small sample size. However, Wallace also found that SR/ST females had substantially higher fecundity than non-carrying ST females. Contrary to these studies, Curtsinger and Feldman (1980) found little evidence of fecundity costs to SR/SR females. One potential criticism of these previous studies is that they used well-established laboratory populations, and differences between fly populations may explain the differences in their results: if a study by chance compared an SR^x chromosome against a particularly high fecundity ST chromosome, then carrying a SR^x may appear costly. In contrast, if the ST chromosome were low fecundity, or carried a high load of deleterious recessive alleles, then SR^x carrying females may appear to have a higher relative fecundity, or heterozygote females might have the highest fecundity, as seen in Wallace's (1948) and Curtsinger and Feldman's (1980) studies. In this study and previous studies, overall female fecundity varies slightly between backgrounds. Indicating that this may be one reason for the inconsistency of past findings, and highlighting the importance of examining the impact of SR^x across multiple genetic backgrounds.

A recent model examining the co-evolutionary dynamics of polyandry and sex ratio drive (Holman et al. 2015), indicates that polyandry alone is insufficient to control the spread of X linked meiotic drivers when allowing female mating behaviour to co-evolve with the frequency of SR^x . This in part stems from meiotic drivers spreading very rapidly through a population (Burt and Trivers 1996). Therefore, polyandry will often fail to evolve rapidly enough to reach a sufficiently high level to stop or reverse the spread of SR^x . However, the models predict that a combination of polyandry with substantial costs to homozygote female SR^x carriers can be enough to stabilize the frequency of drive and prevent fixation. The suggestion that costs to homozygote females prevent the spread of meiotic drivers has been supported by findings in other species; t haplotypes in mice, (Ardile 1998) and driving X chromosomes in *Drosophila. recens* (Dyer et al. 2007).

Previous empirical work has examined the costs of carrying SR in male *D. pseudoobscura*. SR^x males only produce half the number of sperm compared to non-drive males and, as a consequence, are poor sperm competitors only siring ~14% of offspring when competing with an ST male (Price et al. 2008). The disadvantages that SR^x infers in males, promotes females to evolve higher mating frequency and rate in laboratory populations (Price et al. 2010). These studies therefore suggested that polyandry could regulate the frequency of SR^x in natural populations and prompted the creation of Holman's models. Further evidence shows that SR^x occurs in a geographical cline across the USA, being absent or at <1% in northern populations and

increasing in frequency to 20-30% in the southern USA (Wallace 1948, Edwards 1961 and Price et al. 2014). This geographical cline correlates with the frequency of polyandry in the wild, with female multiple mating being more frequent in northern populations, further suggesting that polyandry may regulate the spread of SR^X in nature (Price et al. 2014). This empirical evidence therefore clearly shows an association between polyandry and SR^X , whereas theoretical predictions show that polyandry alone is not sufficient to directly regulate the frequency of SR .

Previously it has been stated that the SR^X chromosome has little consistent effect in females (Powell 1997). Here it is demonstrated that *D. pseudoobscura* females homozygote for SR^X have consistently reduced fecundity. Equally, under the assumption that the failure rate suffered by SR/SR (23%) females is realistic, our findings suggested that there might also be serious sterility costs to females carrying SR^X . Sterility combined with reduced fecundity represents a large fitness cost to female carriers. These results therefore corroborate the recent model prediction, which stipulates that costs to homozygote females are necessary to allow polyandry to effectively regulate the frequency of SR^X in natural populations (Holman et al 2015). Equally our findings are further supported by empirical evidence that SR^X is lower in highly polyandrous populations in the wild (Price et al. 2014), and that polyandry can directly regulate SR^X in laboratory populations (Price et al. 2010).

In conclusion, I found that there were consistent and substantial fecundity costs to SR/SR females across multiple genetic backgrounds. Fecundity costs

have been predicated to be critical to allow polyandry to regulate SR^x (Holman et al. 2015). This suggests that the frequency of SR^x in natural *D. pseudoobscura* populations is indeed stabilised by a combination of polyandry, which results in sperm competition that drive-bearing males lose, and significant costs to homozygous drive females, which inherently increase in costliness whenever drive increases in frequency.

Chapter 4 General Discussion

In this masters thesis I have examined the role of conflict and competition in shaping insect mating systems by focussing on two aspects integral to determining reproductive fitness, namely male-male competition in moths and conflict that stems from selfish genetic elements that distort the sex ratio in flies.

Main findings

Male-male competition in moths

These results reveal large variation in male mating success in a competitive scenario, reflective of the importance of male-male competitive ability in *P. interpunctella*. The moths in this experiment have been evolving under a male biased adult sex ratio for >100 generations, and hence being continuously exposed to high male-male competition. Despite this long history of competition there is still substantial variation in male mating success, suggesting that mating is non-random. The large variation in mating success found in this study, relates to a more general pattern of increased costs on resources invested in spermatophore production and mating activity by males in the MB populations, as well as associated courtship costs. Virgin male *Pieris napi* butterflies that are prevented from mating for example, but still allowed to court unreceptive females show a reduced lifespan of similar magnitude to males with access to receptive mates and allowed to both mate and court (Wedell 2010). Equally, male *Drosophila melanogaster* show reduced immune function when housed with four female flies, as courting and

mating efforts are then increased (McKean and Nunney 2001). The results of this study confirm that large variation in mating success is non-random, with quicker developing and longer living males gaining significantly more copulations over a three-day period in a competitive environment. These findings corroborate previous research in this moth population: that male mating success is associated with rapid development time and longer lifespan (but is not influenced by a difference in body size) when mating under an equal (Lewis et al. 2011) or male biased sex ratio (Willis 2015). These results indicate that development time and longevity are under strong directional selection in male *P. interpunctella*. The fact that this result was revealed here when males were exposed to male-male competition, but also shown previously without mating without male-male competition (Lewis et al. 2011; Willis 2015), indicates that the patterns found in this study are applicable across multiple mating contexts.

One of the benefits of mating the moths in a competitive scenario is that it provides a more accurate measure of a male's ability to gain multiple matings. My results demonstrate that at a competitive male biased sex ratio, 14% of males mate multiply and 3% gain copulations on each of the three days. After showing that there is large variation in male mating success, despite a history of selection for high male mating success, I wanted to examine what factors could potentially maintain this variation. One possibility is a trade-off between development time and lifespan however, this trade-off is not a pattern found in my data (Chapter 2). Nevertheless, results indicate that highly successful males appear to show differences in life-history traits compared to moderately

successful males, with males that gained three copulations developing slower and having reduced adult longevity, which may be sufficient to maintain variation in copulation success in *P. interpunctella*. Overall, these results highlight the importance of considering different types of males i.e. unsuccessful males whose genes are removed from the gene pool and, amongst the successful group, males that are either moderately (mating once in three days) or very successful (mating three times) in gaining copulations. My results reveal that amongst successful males different trait combinations may maintain variation in these life-history traits that are known to be associated with mating success. Maybe this is a more general phenomenon and the differences in trait combinations between successful and very successful males is a factor that needs to be considered more regularly when measuring factors relating to male mating success.

This study only examined pre-copulatory male-male competition. However, clearly sperm competition post-mating plays a role in determining overall reproductive success of males (Pischedda and Rice 2012). A future study examining the combined effects of pre- and post-copulatory mating in a competitive scenario and how these episodes of selection contribute to the overall reproductive success of males would be of interest. Many of the mating assays in previous studies on this moth population, mentioned in this thesis, were performed in absence of any male mating competition (Ingleby et al. 2010, Lewis et al. 2011, Willis 2015). Although it is important to note that the same pattern was found both with and without male-male competition, with regard to the measured life history traits, suggesting the observed pattern

is consistent across mating contexts. However, my experimental design provides a more realistic measure of male-male competition and mate-choice, reflecting the conditions that the moths have evolved under.

Intragenomic competition in flies

My aim was to investigate the importance of potential costs to carrying the meiotic driving sex ratio distorter SR present on the X chromosome in female *Drosophila pseudoobscura* (SR^x). The results of my study quantify the cost of carrying driving SR^x chromosomes for both heterozygote and homozygote females by comparing the lifetime fecundity of females carrying 0, 1 or 2 copies of SR^x. I found evidence of significant fecundity costs to females carrying 2 copies of SR^x. Homozygous SR/SR females produced fewer than half the number of offspring compared to heterozygous SR/ST and standard ST/ST females. Equally, sterility was shown to be high, with 23% of mated SR/SR females producing no offspring in spite of successful copulation. Furthermore, deviations from 1:1 sex ratio were found in offspring being significantly skewed towards daughters with SR/SR (61%) and SR/ST (60%) females producing significantly more female-biased broods. These fecundity, fertility and sex ratio results were consistent across 4 isolines from two different geographic areas.

Taken together, these results indicate that in natural populations there are significant costs to females of harboring SR^x sex ratio chromosomes. It has previously been suggested that polyandry may be effective in regulating SR by promoting sperm competition that undermine the transmission of SR due to

poor sperm competitive ability of SR-carrying males (Price et al. 2010, 2014). However, Holman et al. models (2015) indicate that polyandry alone is insufficient to control the spread of X-linked meiotic drivers. The models predict that a combination of polyandry with substantial costs to homozygote female SR^x carriers is required to stabilize the frequency of meiotic drive and prevent fixation in populations. Previous research has focused on the importance of costs to SR males in stabilising SR (Powell 1997, Price et al. 2008), and previous studies examining the impact of carrying SR^x on female fecundity found varied and contradictory effects (Wallace 1948, Edwards 1961 and Beckenbach 1983). My results on the other hand provide evidence across several genetic backgrounds demonstrating that SR^x is generally detrimental in terms of reduced female fecundity.

The results discussed here thus corroborate the predictions of the Holman et al. (2015) model, which showed that costs to homozygote females are necessary to allow polyandry to effectively regulate the frequency of SR in natural populations. These predictions are also supported by empirical evidence showing that SR is lower in highly polyandrous populations in the wild (Price et al. 2014), and that polyandry can directly regulate SR in laboratory populations (Price et al. 2010). These results have potentially large implications to the field as they corroborate Holman's predictions and suggest that low fecundity of SR/SR females, along with polyandry, appear to regulate the frequency of SR^x in natural populations. Moreover, reductions in fecundity may be a general phenomenon for female carriers of meiotic drivers, and future studies should explore if a combination of reduced female fecundity and polyandry may control the spread of meiotic drivers also in other taxa. A wide

range of selfish genetic elements possibly provides the critical combination of low sperm competitive ability and low fitness in males that could favour polyandry. Selfish elements are ubiquitous in living organisms and frequently compromise male fertility (Price et al. 2008) moreover; we have shown them to seriously compromise female fertility. Therefore SGE's may provide a generally overlooked explanation for why polyandry is very widespread in organisms.

More specific future work on these flies could focus on the frequency of polyandry in *SR/SR* flies. It would be interesting to know whether female multiple mating increases the fecundity of *SR/SR* females. It may be expected that polyandry regulates the frequency of *SR/SR* females, leading to a higher offspring count and adding further weight to the model by Holman et al. (2015). It would also be interesting to test additional fly genotypes from populations either with or without the SR^x allele and see whether there are also genetic differences in fecundity, as has been shown for polyandry (Price et al. 2014), as SR^x is expressed at very different frequencies in the two populations. In this experiment I found slight fluctuations but no significant differences in female fecundity between isolines from the two populations. However, it would be insightful to test more genotypes as a more reliable measure because a higher proportion of polyandry in the Lewiston flies would be expected where SR^x does not naturally occur, and therefore a better ability to regulate the spread of SR^x than the less polyandrous southern Show Low populations, where SR^x occurs naturally.

General conclusion

Overall this thesis has concentrated on sexual selection in moths, with a particular focus on male-male competition over mating. Whereas the study on flies has concentrated on competition arising between the selfish genes that promote their own transmission and the rest of the genome, specifically focusing on potential costs to females. This thesis is, in essence, about maintenance of high male mating success in the face of directional selection in moths and maintenance of a sex ratio distorter in flies. Here I have looked at two different mating systems that are under strong selection, to determine how variation in mating success and a sex ratio distorter can be maintained.

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